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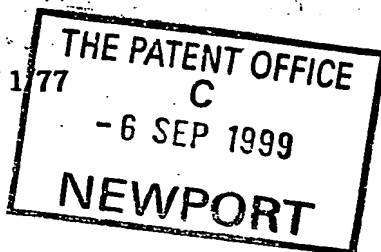
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The Patent Office

Cardiff Road
Newport
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NP9 1RH

6-a-99

1. Your reference

PA9946

2. Patent application number

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9920924.9

3. Full name, address and postcode of the or of each applicant (underline all surnames)

AMERSHAM PHARMACIA BIOTECH UK LTD
Amersham Laboratories
White Lion Road
Amersham
Bucks HP7 9LL. GB

Patents ADP number (if you know it)

If the applicant is a corporate body, give the country/state of its incorporation

GB

762720001

4. Title of the invention

MIXING CHAMBER

5. Name of your agent (if you have one)

Dr Anthony John ROLLINS

"Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode)

Nycomed Amersham plc
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Patents ADP number (if you know it)

7395298001

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and (if you know it) the or each application number

Country

Priority application number
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Date of filing
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7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application

Date of filing
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8. Is a statement of inventorship and of right to grant of a patent required in support of this request? (Answer 'Yes' if:

- a) any applicant named in part 3 is not an inventor, or
- b) there is an inventor who is not named as an applicant, or
- c) any named applicant is a corporate body.

NO

See note (d))

Patents Form 1/77

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Continuation sheets of this form

Description 7

Claim(s) 2

Abstract 1

Drawing(s) 4 + 4 + 4

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Priority documents

Translations of priority documents

Statement of inventorship and right to grant of a patent (Patents Form 7/77)

Request for preliminary examination and search (Patents Form 9/77) 1

Request for substantive examination (Patents Form 10/77)

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11.

I/We request the grant of a patent on the basis of this application.

Signature

A.T. Rollins

Date

31/9/99

12. Name and daytime telephone number of person to contact in the United Kingdom

ANTHONY JOHN ROLLINS 01494 543090

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Mixing Chamber

The present invention relates to dispensing liquid samples containing
5 particles in liquid suspension. In particular the invention relates to a mixing
chamber for mixing and maintaining cells, beads, or other particles in liquid
suspension and to a method for mixing and maintaining liquid suspensions
of cells, beads, or other particles whilst they are being pipetted by means of
a robotic workstation.

10 It is common in the fields of biochemistry and molecular biology to
perform tests in liquid media using particulate samples such as cells and
beads. For example, scintillant-containing beads are used in automated
high throughput screening assays such as radio-immunoassays, receptor
15 binding assays and enzyme assays. There are numerous publications,
which describe such techniques, in which receptors or other binding agents
are immobilised onto the surface of the bead, and are used in the
quantitation of analytes, the detection of competitive binding, or in the
measurement of enzyme activity. Likewise, cells may be grown in liquid
20 suspension and thereafter treated with active agents such as putative or
candidate drug molecules for *in-vitro* drug metabolism, toxicology and drug
profiling studies. In order to automate screening assays it is necessary to
have a means of dispensing beads, cells or other particulate materials into
microwell plates. A number of vendors supply liquid handling
25 instrumentation which can be used to dispense such samples, including
dispensers which can simultaneously dispense into 96- and 384-microwell
plate formats. However, particulate samples must be maintained in an
homogenous suspension to ensure precise dispensing. Conventional
apparatus for mixing fluid suspensions of particulate materials rely on a
30 variety of mechanical mixing arrangements. These include the commercially
available paddle style mixers and rotary tumble style stirrers.

US Patent No.4812856 discloses a method and apparatus for dispensing a fluid with dispersed particles using the ink jet principle, the apparatus comprising a reservoir to hold the fluid and dispersed particles
5 and means to agitate the fluid to maintain the dispersed particles in suspension.

The present invention seeks to provide a mixing chamber which is suitable for mixing particulates in liquid suspension and for dispensing
10 homogeneous samples into microwell plates, which is an alternative to current apparatus and methods and which can be used with a range of particulate sizes and types, both biological and non-biological.

According to one aspect of the present invention, there is provided
15 apparatus for mixing and maintaining particulates in liquid suspension, the apparatus comprising a reservoir for holding a fluid containing dispersed particles, a substantially horizontally-disposed mixing plate mounted inside the reservoir, the mixing plate having a plurality of vertical holes extending
through the plate, and means for raising and lowering the mixing plate
20 relative to the reservoir.

Suitably, the mixing plate contains an array of holes extending through the plate, the holes being in fixed relationship one with another. Preferably the mixing plate contains an array of 24-, 96- or 384-holes.
25

The reservoir is mounted inside an outer casing. Preferably, the means for raising and lowering the mixing plate inside the reservoir comprises at least one piston in contact with the outer casing of the apparatus and operatively connected to an air supply. The air supply to the
30 piston (not shown) is controlled by means of a pressure regulator, solenoids and throttle valve so as to deliver bursts or pulses of air to activate the

piston. Alternatively, the mixing plate may be raised and lowered by an electrically driven motor, by a solenoid mechanism, by hydraulic pressure activating a piston, or by a magnetic mechanism.

5 Preferably, the apparatus further comprises means for adding samples in liquid suspension to the reservoir by means of a reagent feed pipe and means for removal or re-circulation of unused liquids from the reservoir by means of an overflow pipe.

10 In a second aspect, the invention provides a method for mixing and maintaining particulate materials in liquid suspension, using the apparatus as described, the method comprising the steps of introducing a liquid suspension of particulate materials to the mixing chamber reservoir, actuating the mixing plate inside the reservoir so as to mix and maintain the
15 particulate materials in liquid suspension.

 Suitably, the particulate materials may be selected from biological or non-biological sources, for example, cells (eukaryotic cells, prokaryotic cells), viral particles, glass beads, scintillant beads (PVT, polystyrene,
20 yttrium silicate, yttrium oxide), magnetic latex beads, chromatography media, controlled pore glass beads, and the like.

 In order to clarify the principle and the function of the invention, reference is now made to the accompanying drawings in which:

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 Figure 1 shows a plan view of the apparatus (10) according to the invention, including the reservoir (20), the mixing plate (30) and the outer casing (40) components of the apparatus.

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 Figure 2 shows a plan view (Figure 2a) and side elevations (Figures 2b and 2c) of the reservoir (20) according to the invention.

Figure 3 shows a plan view (Figure 3a) and side elevation (Figure 3b) of the mixing plate (30) according to the invention.

5 Figure 4 shows a plan view (Figure 4a) and side elevations (Figures 4b and 4c) of the outer casing (40) according to the invention.

Referring to Figures 1-4 of the drawings, the apparatus (10) for mixing and maintaining particulates in liquid suspension includes a reservoir (20) (shown in Figure 2) for holding a fluid sample containing dispersed particles. The reservoir (20) is rectangular in cross-section, having an open top, side walls (21a, 21b), end walls (22a, 22b), and a base (23). The reservoir (20) is sized to accommodate the substantially horizontally-disposed mixing plate (30) such that there is free vertical movement of the mixing plate within the reservoir (20). Connected vertically to the centre of each end wall (22a, 22b) of the reservoir (20) are linear guide rails (24), each of the linear guide rail mechanisms being supplied with points for holding the outer casing (40) in registry with the reservoir for guiding the outer casing up and down in relation to the reservoir (20).

20

The reservoir includes a horizontally disposed bracket (25) extending from one side wall (21b), into which is set vertically a piston (26) for raising and lowering the outer casing of the apparatus. The piston may be positively located to the outer casing by means of a dome-headed locking nut connected to the exposed thread of the piston, in which case retraction of the piston serves to draw the outer casing down over the reservoir in a vertical movement. In the alternative, the piston is not positively connected to the outer casing, in which case, the casing is lowered by gravitational force. As stated above, in a preferred embodiment of the invention, the piston is actuated so as to raise and lower the outer casing (40) and mixing plate (30) components of the apparatus by means of

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an air supply. Control of the air supply to actuate the mixing plate is made possible by well-known means. The control of the stroke on the mixing plate is by an electrical timer pulsing a solenoid valve to open at predetermined intervals. The air is throttled to alter the severity of the stroke before outputting to the piston. Alternatively, the mixing plate may be raised and lowered by hydraulic pressure activating the piston, or electrically, either by means of a motor or by a solenoid.,

Preferably the apparatus has means for adding particulate materials in liquid suspension to the reservoir through a reagent feed pipe (27) set into the lower portion of one end wall (22a) of the reservoir and means for removal or re-circulation of unused liquid samples from the reservoir through a pipe (28) under the control of an outlet valve (not shown). In use, reagents or wash solutions may be added to the reservoir by means of a syringe connected to the reagent feed pipe, or by means of a peristaltic pump connected to a second reservoir, or by gravity. Reagents may be removed from the reservoir by means of a suction pump connected to the waste pipe. Preferably the apparatus includes an overflow pipe (29) set into either end wall (22a, 22b) of the reservoir (20).

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Suitably the reservoir (20) is of a one-piece moulded construction and may be formed from rigid materials, which are resistant to the aqueous, and/or aqueous/organic media used in the dispensing operation. Suitable materials are selected from stainless steel and rigid plastic or polymeric materials. Alternatively the reservoir may be formed by machining methods in the surface of a plastic or polymeric block. Preferred plastics are selected from polystyrene, polycarbonate, acetal (polyoxymethylene homopolymer or co-polymer), or poly-tetrafluoroethylene (PTFE; Teflon™).

30

The mixing plate is shown in Figure 3 and comprises a solid rectangular sheet or block (30) with side walls (31), end walls (32), a base

(33) and a top (34), wherein there are formed in the block an array of vertical holes (35) extending through the block from the top surface to the base. As stated previously, the mixing plate is sized to fit inside the reservoir such that there is free vertical movement of the plate within the reservoir (20). Located in holes (36) drilled into the top surface (34) at each end of the mixing plate are suspension handles (37) secured by a friction fit into the holes. The suspension handles are formed from stainless steel and extend upward to connect with lugs located on the outer casing (40) of the apparatus.

Referring to Figure 3, a 384-hole plate (30) is shown having the dimensions 12.8 x 8.6 x 0.5cm with the 384 holes in an array of 16 rows x 24, spaced apart such that the centre of each hole in the plate is precisely located to coincide with the vertical axis of each of the corresponding wells of a 384-well microplate. The mixing plate may be constructed to contain 24-, 96-, or 384-holes as required for dispensing particulate samples into 24-well, 96-well or 384-well microplates. Each of the holes in an array is of substantially the same dimension and is suitably of a diameter which will allow a pipette-tip to be inserted through the hole in the mixing plate into the sample contained in the reservoir. The diameter of each of the holes in the array may be from 2mm to 7mm, preferably from 2mm to 4mm. The diameter of each of the holes in a 384-hole array is preferably 3mm. Suitably, the mixing plate is fabricated from a rigid plastic material, preferably acetal (polyoxymethylene homopolymer or co-polymer), or polytetrafluoroethylene (PTFE; Teflon™).

The outer casing (40) of the apparatus is shown in Figure 4 and is sized to fit over the reservoir (20), including the guide rails (24), and piston (26), while allowing free access of a multi-head pipetter to the sample contained in the reservoir. The outer casing is formed in rectangular cross-section with side walls (41a, 41b), end walls (42a, 42b) and a flange (44)

extending horizontally inwards from the vertical walls of the casing.

Connected to the flange at each end wall of the casing (40) are lugs (45) suitable for engaging with the suspension handles (37) of the mixing plate (30), for raising the mixing plate. The end walls may include one or more

5 slots (46) to allow reagent and waste feed pipe access to the reservoir (20).

Extending horizontally outwards from the side wall (41b) is a bracket (47) to which may be connected inside the piston for upward movement of the casing (40). The casing is mounted over the reservoir and connected to the guide rails (24) by fixing means, such as screws passing through the outer
10 casing in each end wall.

Suitably the outer casing (40) of the apparatus is of moulded construction and may be formed from rigid materials which are resistant to the aqueous and/or aqueous/organic media used in the dispensing
15 operation. Suitably the outer casing is fabricated from stainless steel.

In operation a liquid suspension of particulates is introduced into the reservoir of the assembled apparatus, by means of the reagent feed pipe. The air supply is switched on to actuate the piston at predetermined
20 intervals, causing the upward movement of the casing and mixing plate, thereby agitating and mixing the particulates in suspension. Upon relaxation of the air pressure the outer casing (and mixing plate) are drawn down (or are allowed to return to their original position under gravity) in readiness for the next cycle.

25 By the use of the mixing chamber formed in accordance with the invention and actuating the mixing plate so as to raise and lower the mixing plate, it is possible to mix and maintain particulates in liquid suspension and to remove aliquots of particulate materials in liquid suspension before the
30 materials have had a chance to settle out in the reservoir.

Claims

1. Apparatus for mixing and maintaining particulates in liquid suspension, the apparatus comprising a reservoir for holding a fluid
5 containing dispersed particles, a substantially horizontally-disposed mixing plate mounted inside the reservoir, the mixing plate having a plurality of vertical holes extending through the plate, and means for raising and lowering the mixing plate relative to the reservoir.
 - 10 2. Apparatus as claimed in claim 1 wherein the reservoir further comprises means for adding samples in liquid suspension to the reservoir by means of a reagent feed pipe and means for removal or re-circulation of unused liquids from the reservoir by means of an overflow pipe.
 - 15 3. Apparatus as claimed in claims 1 or 2 wherein the mixing plate contains an array of holes extending through the plate, the holes being in fixed relationship one with another.
 4. Apparatus as claimed in claim 3 wherein the mixing plate contains an
20 array of 24-, 96- or 384-holes.
 5. Apparatus as claimed in any of claims 1-4 wherein the means for raising and lowering the mixing plate inside the reservoir comprises at least one piston in contact with the outer casing of the apparatus and operatively
25 connected to an air supply.
 6. A method for mixing and maintaining particulate materials in liquid suspension, using the apparatus according to any one of claims 1-5, the method comprising the steps of introducing a liquid suspension of
30 particulate materials to the mixing chamber reservoir, actuating the mixing
-

plate inside the reservoir so as to mix and maintain the particulate materials in liquid suspension.

7. The method as claimed in claim 6 wherein the particulate materials are selected from cells (eukaryotic cells, prokaryotic cells), viral particles, glass beads, scintillant beads (PVT, polystyrene, yttrium silicate, yttrium oxide), magnetic latex beads, chromatography media, and controlled pore glass beads.

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Abstract

An apparatus is provided for mixing and maintaining particulates in liquid suspension, the apparatus comprising a reservoir for holding a fluid
5 containing dispersed particles, a substantially horizontally-disposed mixing plate mounted inside the reservoir, the mixing plate having a plurality of vertical holes extending through the plate, and means for raising and lowering the mixing plate inside the reservoir.

Figure 1

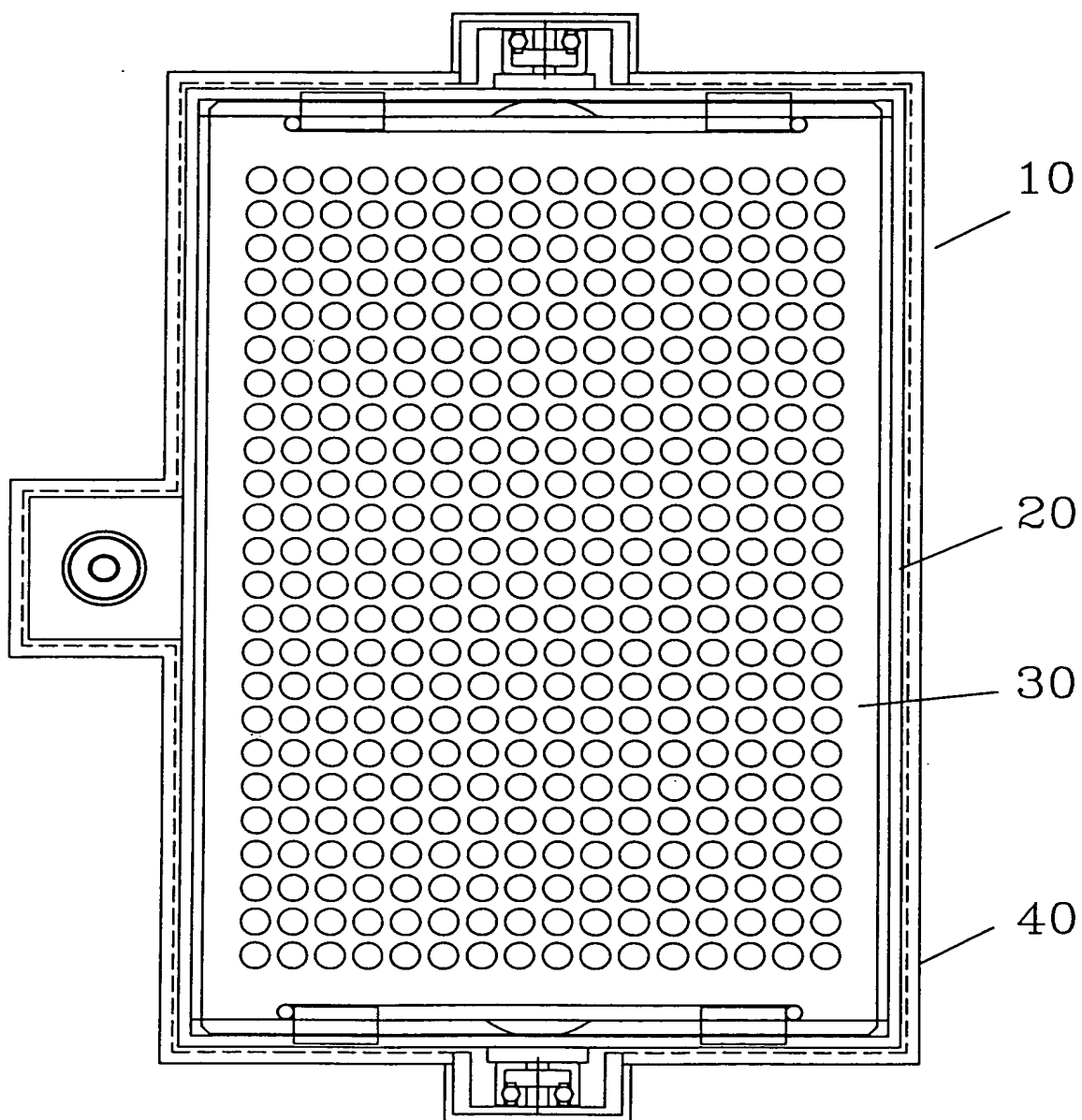


Figure 2

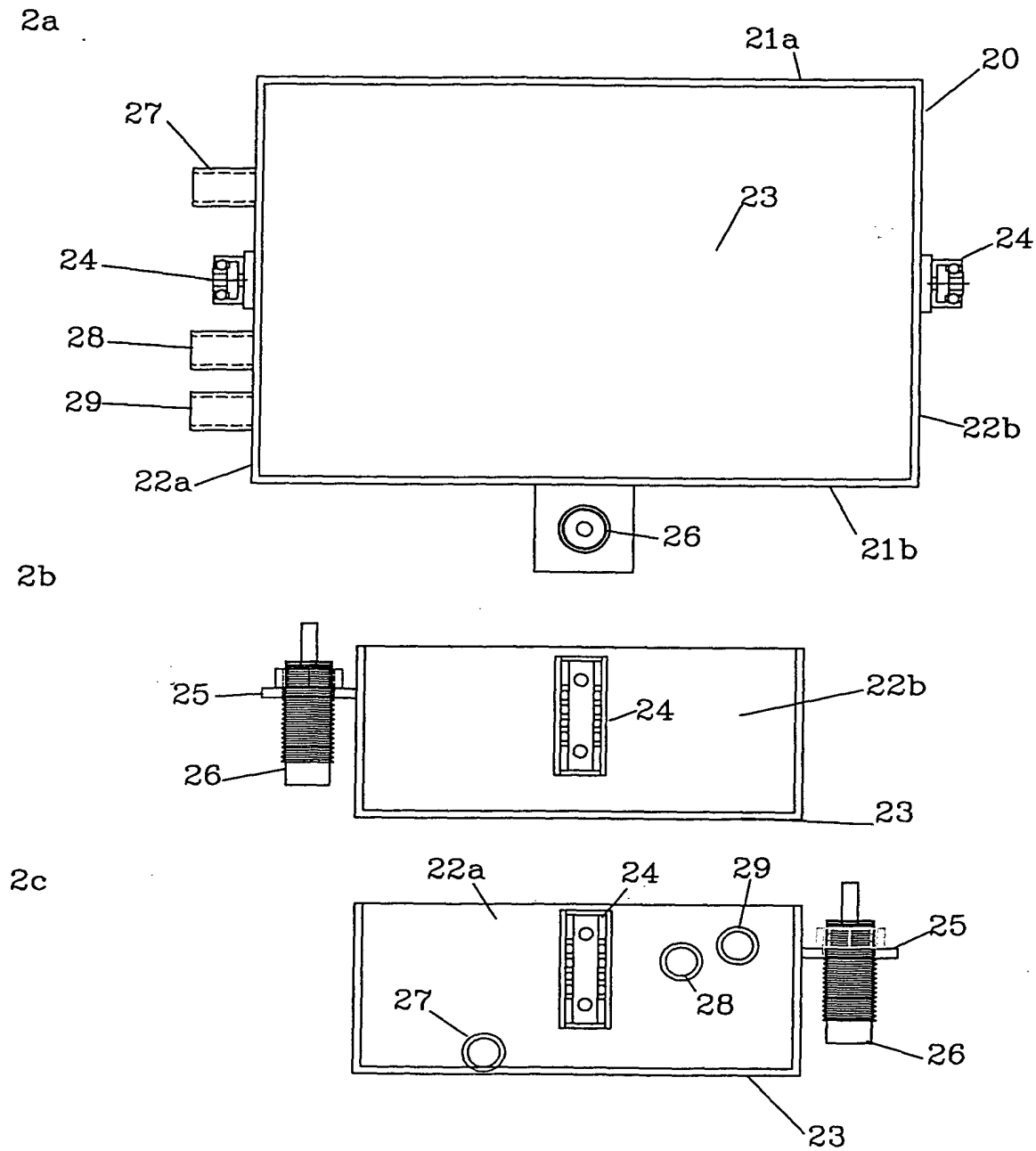


Figure 3

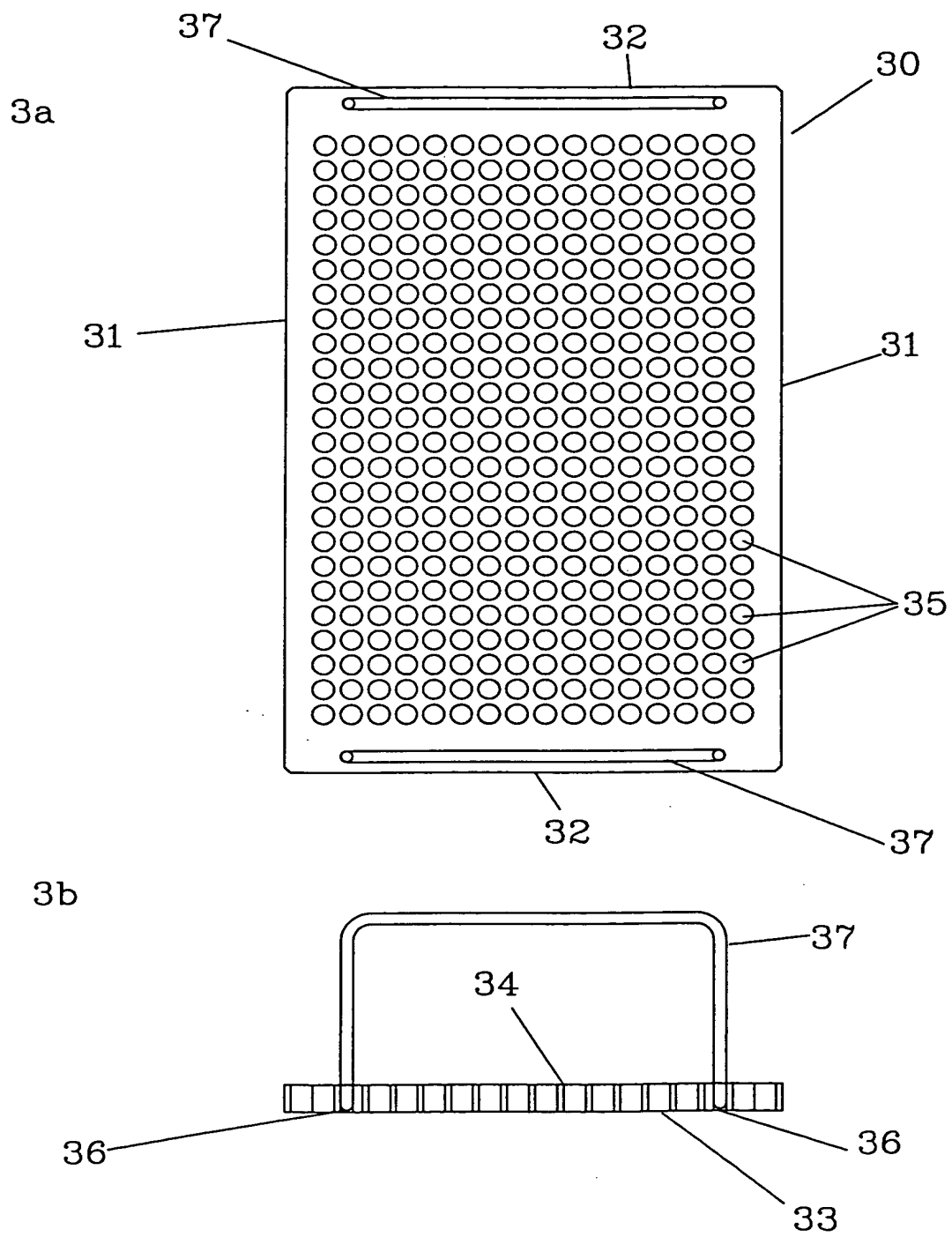
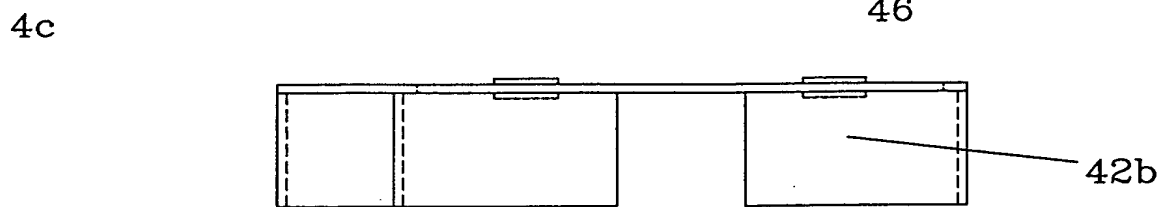
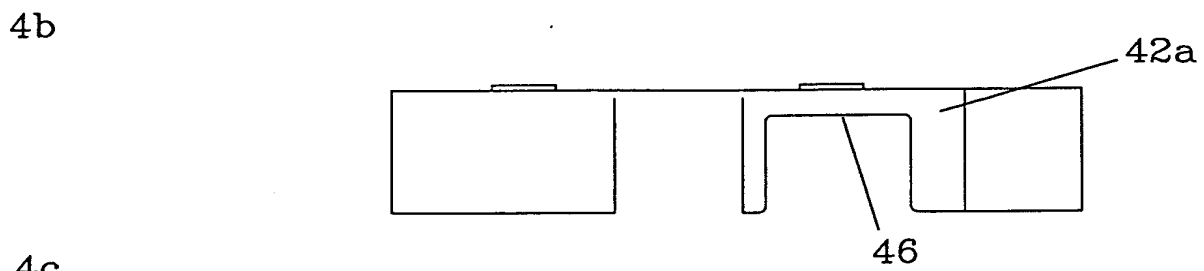
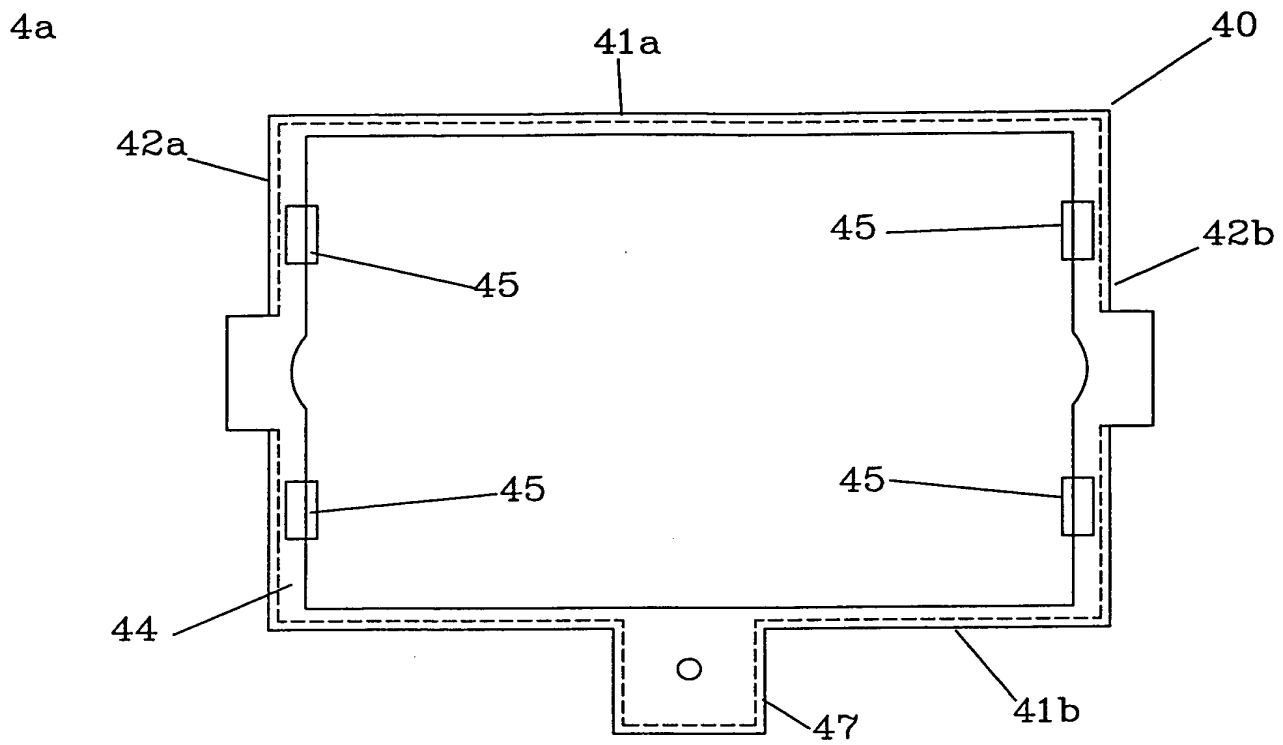


Figure 4





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PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference PA9946-PCT	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416) FOR FURTHER ACTION	
International application No. PCT/GB00/03346	International filing date (day/month/year) 01/09/2000	Priority date (day/month/year) 06/09/1999
International Patent Classification (IPC) or national classification and IPC G01N33/53		
Applicant AMERSHAM PHARMACIA BIOTECH UK LIMITED		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.


2. This REPORT consists of a total of 5 sheets, including this cover sheet.

- ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☒ Certain defects in the international application
- VIII ☒ Certain observations on the international application

Date of submission of the demand 26/03/2001	Date of completion of this report 27.07.2001
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